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# Effect of cigarette smoking on cough reflex induced by TRPV1 and TRPA1 stimulations

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## KEYWORDS

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## Summary

**Background:** Recent studies have shown that neurogenic inflammation induced by cigarette smoke is inhibited by TRPA1 antagonist, but not by TRPV1 antagonist. Since cough reflex sensitivity is known to be modified by smoking status, we investigated the effects of cigarette smoking on TRPA1- and TRPV1-induced cough and urge-to-cough in healthy males.

**Methods:** Twenty-six healthy never-smokers and 30 healthy current smokers were recruited via public postings. Cough reflex thresholds and urge-to-cough were evaluated by inhalation of capsaicin, a TRPV1 agonist, and cinnamaldehyde, a TRPA1 agonist. The cough reflex thresholds were defined as the lowest concentrations of capsaicin and cinnamaldehyde that elicited two or more coughs ( $C_2$ ) and five or more coughs ( $C_5$ ), respectively. The urge-to-cough was evaluated using the modified Borg scale.

**Results:** In capsaicin-induced cough, the cough reflex thresholds, as expressed by  $C_2$  and  $C_5$ , in current smokers were significantly higher than those in never-smokers ( $p < 0.01$  and  $p < 0.001$ , respectively). The urge-to-cough log–log slopes in current smokers were significantly lower than those of never-smokers ( $p < 0.001$ ). There were no significant differences in the thresholds of the urge-to-cough between never-smokers and current smokers. In cinnamaldehyde-induced cough, there were no significant differences in cough reflex thresholds in  $C_2$  and  $C_5$  between never-smokers and current smokers, nor were there any significant differences in urge-to-cough log–log slope between never-smokers and current smokers. There were no significant differences in the thresholds of the urge-to-cough between never-smokers and current smokers.

**Conclusion:** The study suggests that smoking has a differential effect on cough responses between TRPV1 and TRPA1 stimulations.

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## Introduction

Cigarette smoke is one of the most common inhaled irritants of the respiratory tract. In never-smokers, inhaling only a small puff of cigarette smoke can evoke airway irritation and vigorous cough responses.<sup>1</sup> In addition, cigarette smoking is well known to be a risk factor for chronic cough and chronic obstructive pulmonary disease, which are associated with the symptom of cough.<sup>2</sup> However, several studies have shown that chronic smokers without a history or symptoms of airway disease have a significantly diminished cough reflex sensitivity to capsaicin (they cough less in response to inhaled capsaicin) compared to that of healthy never-smokers.<sup>3–7</sup>

Cough results from the stimulation of sensory receptors within the respiratory tract, the afferent impulses of which activate the brainstem and higher cortical centers for cough. Both TRPV1 (transient receptor potential cation channel, subfamily V, member 1) and TRPA1 (transient receptor potential cation channel, subfamily A, member 1) channels have been implicated in the afferent sensory loop of the cough reflex.<sup>8,9</sup> TRPA1, like TRPV1, is expressed by trigeminal and nodose/jugular ganglia neurons.<sup>10</sup> Both channels are most often found in the same neuron.<sup>11</sup> Although this means that activation of TRPA1 will likely exert effects similar to those observed following the activation of TRPV1,<sup>10</sup> recent studies have shown that neurogenic inflammation induced by cigarette smoking is inhibited by HC-030031, a TRPA1 antagonist, but not by capsazepine, a TRPV1 antagonist.<sup>12</sup>

Although there are various chemical components among the approximately 5000 constituents of cigarette smoke, nicotine, reactive oxygen species, and, unsaturated aldehydes included in cigarette smoke have been found to activate the TRPA1 channel, but not the TRPV1 channel.<sup>12–14</sup> Therefore, these components may lead to up-regulation of peripheral afferent activity for cough induced by TRPA1 stimulation but not by TRPV1 stimulation. However, there has been a report that nicotine acts on central neural mechanisms as an anxiolytic,<sup>15</sup> suggesting that it may cause the down-regulation of the cortical facilitatory pathway for cough.

The cough reflex thresholds and perception of urge-to-cough in response to TRPV1 or citric acid stimulations are known to be modified by smoking status.<sup>16,17</sup> However, the effect of cigarette smoking on cough reflex sensitivity to TRPA1 stimulation is unknown. Since TRPA1 plays a role in tobacco smoking-induced neurogenic airway inflammation, we investigated the effects of cigarette smoking on cough reflex thresholds in response to TRPV1 and TRPA1 stimulations. Simultaneously, we demonstrated the effects of smoking on perception of urge-to-cough induced by TRPV1 and TRPA1 stimulations.

## Methods

### Subjects

Twenty-six healthy male never-smokers and 30 healthy male current smokers were recruited to evaluate cough related responses to inhaled capsaicin, a TRPV1 agonist,

and cinnamaldehyde, a TRPA1 agonist. All were recruited via public postings in and around the Tohoku University School of Medicine campus. Never-smoker males and current smoker males were entered into a randomized, crossover study of inhalational cough challenge of two tussive agents. The study was approved by the Institutional Review Board of the Tohoku University School of Medicine. Subjects were without a history of pulmonary and airway diseases, recent (within 4 weeks) suggestive symptoms, respiratory tract infection, and seasonal allergies. Subjects did not take any regular medication.

### Cough reflex thresholds to capsaicin or cinnamaldehyde and urge-to-cough

Cough reflex thresholds in response to capsaicin and cinnamaldehyde were measured on different days using a modification of the methods of capsaicin-induced cough reported by Fujimura et al.,<sup>18</sup> and the cinnamaldehyde-induced cough reported by Birrell et al.<sup>9</sup> The current smokers smoked more than one cigarette within 2 h of each cough challenge. Capsaicin (30.5 mg) (Sigma Aldrich, Seattle, USA) was dissolved in Tween 80 (1 ml) and ethanol (1 ml) and then dissolved in physiological saline (8 ml) to make a stock solution of 0.01 M, which was stored at  $-20^{\circ}\text{C}$ . This solution was diluted with physiological saline to make testing solutions starting at a concentration of  $0.49\text{ }\mu\text{M}$  and increasing it by doubling the concentration up to  $1000\text{ }\mu\text{M}$ . On the other hand, based on the method reported by Birrell et al., cinnamaldehyde (Sigma Aldrich, Seattle, USA) was dissolved in 50% ethanol to make a stock solution of 800 mM.<sup>9</sup> This stock solution was diluted with 50% ethanol to make solutions ranging from 60 to 800 mM.<sup>9</sup>

Each subject inhaled the control solution followed by progressively increasing concentrations of capsaicin or cinnamaldehyde solutions. Subjects inhaled solutions for 15 s every 60 s, by tidal mouth-breathing, while wearing a nose-clip of an ultrasonic nebulizer (MU-32; Sharp Co Ltd; Osaka, Japan).<sup>19,20</sup> The nebulizer generated particles with a mean mass median diameter of  $5.4\text{ }\mu\text{m}$  at an output of 2.2 mL/min. By tidal breathing, one-half of the particles were expected to be deposited in the lungs.<sup>21</sup> The cough reflex threshold and suprathreshold were estimated by the lowest concentrations of capsaicin or cinnamaldehyde that elicited two or more coughs ( $C_2$ ) and the lowest concentrations of capsaicin or cinnamaldehyde that elicited five or more coughs ( $C_5$ ). In a preliminary experiment, we assessed data reproducibility from two consecutive TRPA1-induced cough challenges, spaced 1 week apart. We obtained strong correlations in  $C_2$  and  $C_5$  between the initial application and the second application in eight healthy subjects ( $r = 0.85$ ,  $p < 0.01$ ;  $r = 0.83$ ,  $p < 0.01$ , respectively).

Immediately after the completion of each nebulizer application, the subject made an estimate of the urge-to-cough on the modified Borg scale. The scale ranged from "no need to cough" (rated 0) and "maximum urge-to-cough" (rated 10). The urge-to-cough scale was placed in front of the subjects and the subject pointed at the scale number, which was recorded by the experimenter. To assess the intensity of the urge-to-cough, subjects were recommended to ignore other sensations such as dyspnea,

burning, irritation, choking, and smoke in their throat. Subjects were told that their sensation of an urge-to-cough could increase, decrease, or stay the same during the capsaicin or cinnamaldehyde challenges, and that their use of the modified Borg scale should reflect this.

In each subject, the estimated urge-to-cough scores were plotted against the corresponding capsaicin or cinnamaldehyde concentrations using a log–log transformation. Since it is known that there is a linear relationship between estimated urge-to-cough scores and tussive agent concentration on a log–log scale,<sup>22,23</sup> the slope and intersection were determined by linear regression analysis on a log–log scale.<sup>16,24,25</sup> The thresholds of the urge-to-cough in each subject was estimated as an intersection with the x-axis (citric acid concentration axis), indicating the dose of the urge-to-cough score = 1.<sup>16,24,25</sup> We also determined the initial concentrations of capsaicin or cinnamaldehyde that induced urge-to-cough sensations without provoking associated motor cough events as a threshold of urge-to-cough, termed  $C_U$ .<sup>26</sup>

## Statistical analysis

Data are expressed as mean  $\pm$  SD. The cough reflex threshold was log transformed. The Mann–Whitney  $U$  test was used to compare never-smokers and current smokers variables. A  $p < 0.05$  was considered significant.

## Results

All 56 subjects completed the experiments without any difficulty or side effects. The characteristics of subjects are summarized in Table 1. There were no statistically significant differences in age, height, weight, and spirometric measurements between never-smokers and current smokers.

Figure 1 shows comparisons of cough reflex thresholds in response to capsaicin between never-smokers and current smokers. As shown in Figure 1a, the cough reflex threshold to capsaicin, as expressed by log  $C_2$ , in current smokers ( $1.12 \pm 0.49 \mu\text{M}$ ) was significantly greater than that in never-smokers ( $0.75 \pm 0.41 \mu\text{M}$ ,  $p < 0.01$ ). Similarly, Figure 1b shows that current smokers ( $0.95 \pm 0.36 \mu\text{M}$ ) had

a significant enhancement of log  $C_5$  compared to never-smokers ( $1.42 \pm 0.36 \mu\text{M}$ ,  $p < 0.001$ ).

The log–log slope between capsaicin concentration and the Borg scores of the urge-to-cough were estimated for each subject. As shown in Figure 2a, the urge-to-cough log–log slope in current smokers ( $0.54 \pm 0.27$  point  $\mu\text{M}$ ) was significantly lower than that in never-smokers ( $0.89 \pm 0.31$  point  $\mu\text{M}$ ,  $p < 0.001$ ). The urge-to-cough thresholds in response to capsaicin were estimated as an intersection with the x-axis of the linear regression equation of the log–log relationships between capsaicin concentration and the Borg scores of the urge-to-cough.<sup>16,24,25</sup> As shown in Figure 2b, there were no significant differences in log urge-to-cough threshold induced by capsaicin between never-smokers ( $-0.20 \pm 0.49 \mu\text{M}$ ) and current smokers ( $-0.50 \pm 1.36 \mu\text{M}$ ). We also defined the initial concentration of capsaicin that induced a urge-to-cough sensation without provoking an associate motor cough event, termed  $C_U$ .<sup>26</sup> As shown in Figure 2c, capsaicin-induced log  $C_U$  in current smokers ( $0.21 \pm 0.34 \mu\text{M}$ ) did not differ from that of never-smokers ( $0.25 \pm 0.31 \mu\text{M}$ ). These data indicate that smoking causes no significant changes in bronchopulmonary sensors involved in the urge-to-cough induction induced by capsaicin and that there is a larger contribution of central gain mechanisms than that of peripheral ones.

Figure 3 shows comparisons of cough reflex thresholds to cinnamaldehyde between never-smokers and current smokers. As shown in Figure 3a, cinnamaldehyde-induced log  $C_2$  was similar in never-smokers ( $2.61 \pm 0.30$  mM) and current smokers ( $2.68 \pm 0.20$  mM). Figure 3b shows that cinnamaldehyde-induced log  $C_5$  was also similar in never-smokers ( $2.70 \pm 0.25$  mM) and current smokers ( $2.76 \pm 0.16$  mM).

In the same way as capsaicin, the log–log slope between cinnamaldehyde concentration and the Borg scores of the urge-to-cough was estimated for each subject. Figure 4a shows that the urge-to-cough log–log slope induced by cinnamaldehyde in current smokers ( $0.98 \pm 0.32$  point mM) did not differ from that of never-smokers ( $1.04 \pm 0.51$  point mM). In addition to this phenomenon, there were no significant differences in log urge-to-cough threshold and  $C_U$  between never-smokers ( $1.86 \pm 0.79$  mM and  $2.24 \pm 0.29$  mM, respectively) and current smokers ( $2.08 \pm 0.27$  mM; and  $2.24 \pm 0.28$  mM, respectively).

## Discussion

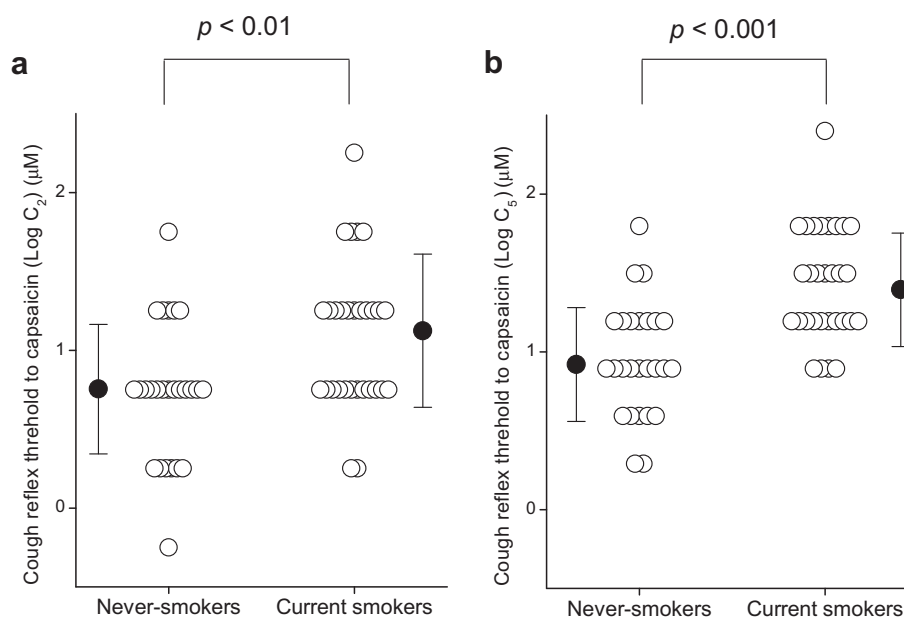
In this study, we found that the increased cough reflex thresholds induced by capsaicin in current smokers were accompanied by decreased urge-to-cough log–log slope, whereas the thresholds for urge-to-cough did not differ between never-smokers and current smokers. In cinnamaldehyde-induced cough, we showed that cough reflex thresholds were not significantly different between never-smokers and current smokers. Simultaneously, urge-to-cough log–log slope, log urge-to-cough threshold and  $C_U$  were similar in never-smokers and current smokers.

There have been several studies concerning the effect of smoking on the cough reflex<sup>3–7,16</sup> which applied capsaicin and citric acid cough challenge methods. In this study,

**Table 1** Comparison of characteristics between never-smokers and current smokers.

Variable	Never-smokers	Current smokers	$P$ value
Number	26	30	—
Age (years)	$26.0 \pm 7.6$	$26.6 \pm 8.5$	n.s.
Height (cm)	$171.6 \pm 5.2$	$173.3 \pm 6.7$	n.s.
Weight (kg)	$66.0 \pm 8.2$	$67.4 \pm 12.0$	n.s.
Brickman index	0	$121.3 \pm 117.1$	—
FEV <sub>1</sub> (L)	$4.10 \pm 0.53$	$4.20 \pm 0.70$	n.s.
FVC (L)	$4.68 \pm 0.71$	$4.81 \pm 0.75$	n.s.
FEV <sub>1</sub> /FVC (%)	$87.4 \pm 5.82$	$87.4 \pm 6.79$	n.s.

Data are mean  $\pm$  SD.  $P$  value by the Mann–Whitney  $U$  test. FEV<sub>1</sub>, forced expired volume in 1 s; FVC, forced vital capacity.

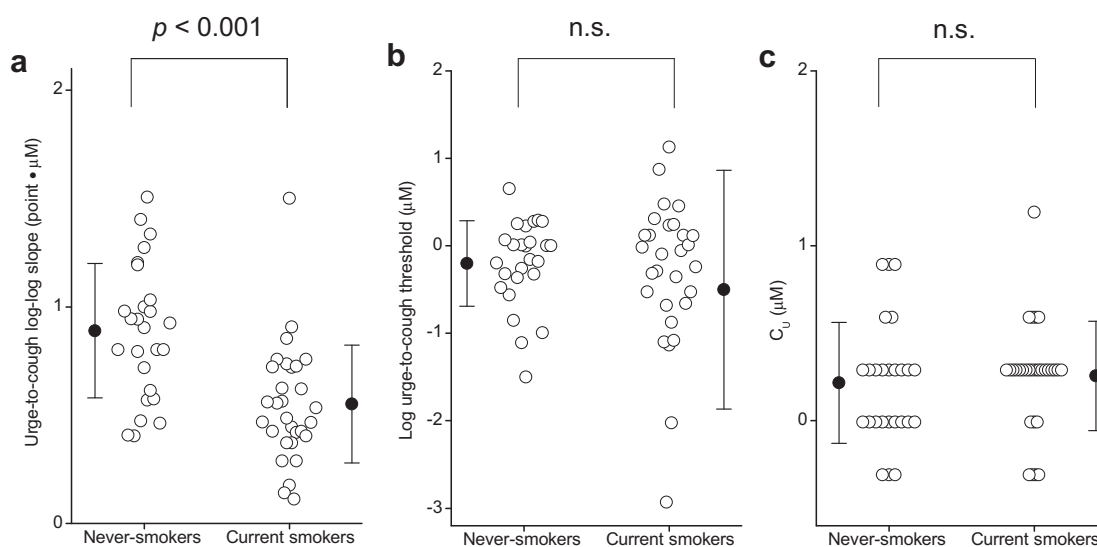


**Figure 1** Comparisons of cough reflex thresholds to capsaicin between never-smokers and current smokers. (a) Cough reflex threshold expressed as the log transformation of the lowest concentration of capsaicin that elicited two or more coughs ( $C_2$ ). (b) Cough reflex threshold expressed as the log transformation of the lowest concentration of capsaicin that elicited five or more coughs ( $C_5$ ). Closed circles and error bars indicate the mean value and the standard deviation in each group, respectively.

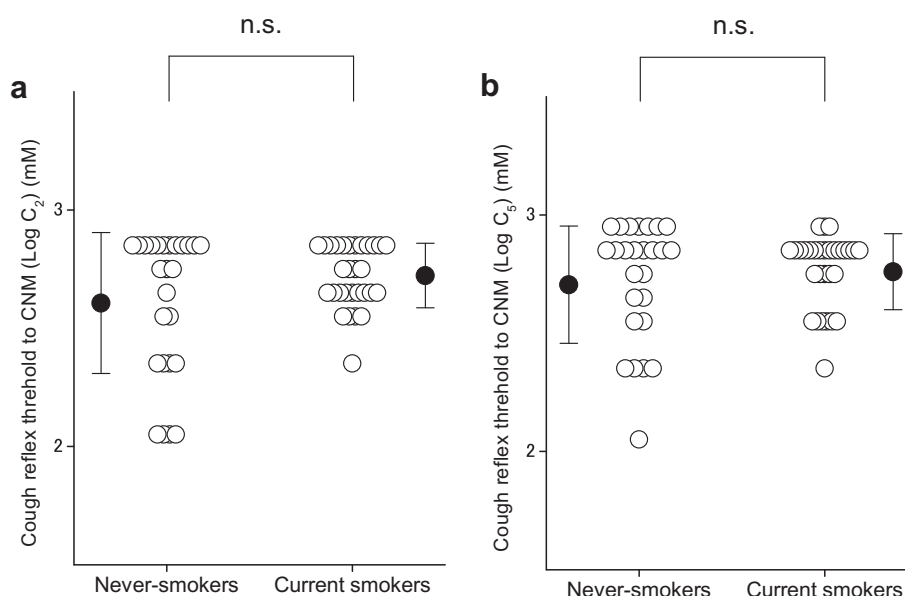
based on the capsaicin-induced cough challenge method reported by Fujimura,<sup>17</sup> we demonstrated that our observations are consistent with past studies which investigated the effect of smoking on cough reflex sensitivity to capsaicin.<sup>3–7</sup>

In contrast, the effect of smoking on cinnamaldehyde-induced cough has not been investigated. There is only one

study, that by Birrell et al.,<sup>9</sup> concerning cinnamaldehyde-evoked coughing in humans. According to ERS guidelines on the assessment of cough,<sup>27</sup> they employed the dosimeter method in assessing cough response to cinnamaldehyde. We, however, used the tidal breathing method. Since there has been a report that the capsaicin cough threshold in the tidal breathing method is a strongly correlated with that in



**Figure 2** Comparisons of urge-to-cough induced by capsaicin between never-smokers and current smokers. (a) The urge-to-cough log-log slope by linear regression between capsaicin concentration and log Borg scores. (b) The urge-to-cough threshold estimated by log capsaicin concentration at the log Borg score of urge-to-cough = 0. (c) The  $C_u$  induced by capsaicin indicates the concentration of capsaicin provoking perception of urge-to-cough without associated motor cough. Open circles indicate the value of each subject. Closed circles and error bars indicate the mean value and the standard deviation in each group, respectively. n.s. denotes not significant.

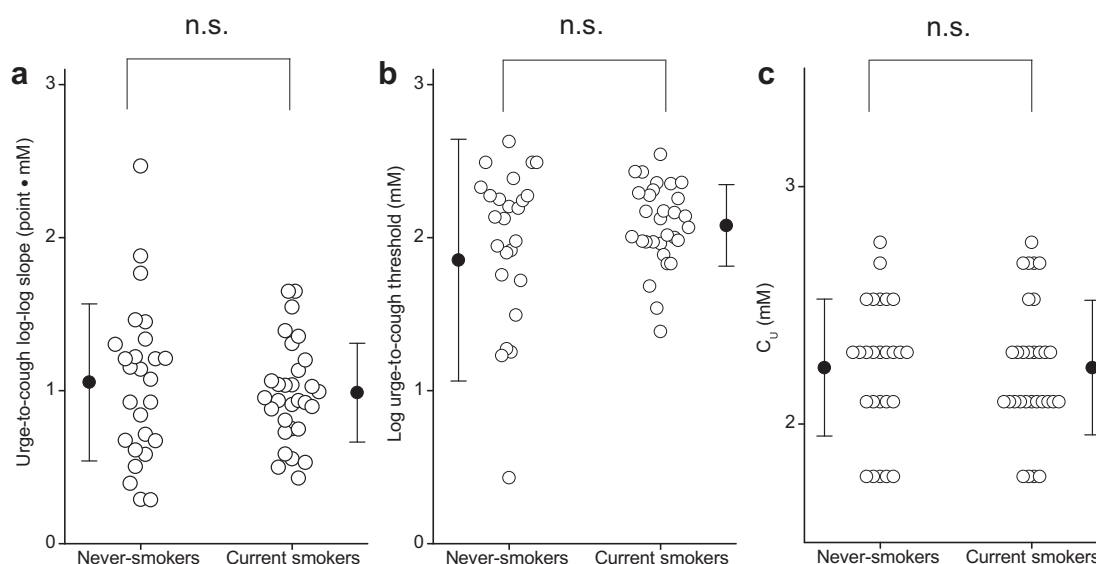


**Figure 3** Comparisons of cough reflex thresholds to cinnamaldehyde (CNM) between never-smokers and current smokers. (a) Cough reflex threshold expressed as the log transformation of the lowest concentration of cinnamaldehyde that elicited two or more coughs ( $C_2$ ). (b) Cough reflex threshold expressed as the log transformation of the lowest concentration of cinnamaldehyde that elicited five or more coughs ( $C_5$ ). Closed circles and error bars indicate the mean value and the standard deviation in each group, respectively.

the dosimeter method,<sup>28</sup> the cinnamaldehyde-induced cough response may also have a strong correlation between two methods. Although we recruited only male subjects in our protocol, Birrell et al. recruited both males and females.<sup>9</sup> Therefore, since the cough reflex is known to be modified by the gender factor,<sup>24</sup> the difference in

cinnamaldehyde-induced cough between our study and the experiment of Birrell et al. probably resulted from the gender factor.

In experimental animals (e.g., guinea pigs), chronic exposure of the airways to cigarette smoke induced cough hypersensitivity to various tussive inhalation challenges. In



**Figure 4** Comparisons of urge-to-cough induced by cinnamaldehyde between never-smokers and current smokers. (a) The urge-to-cough log-log slope by linear regression between cinnamaldehyde concentration and log Borg scores. (b) The urge-to-cough threshold estimated by log cinnamaldehyde concentration at the log Borg score of urge-to-cough = 0. (c) The  $C_0$  induced by cinnamaldehyde indicates the concentration of cinnamaldehyde provoking perception of urge-to-cough without associated motor cough. Open circles indicate the value of each subject. Closed circles and error bars indicate the mean value and the standard deviation in each group, respectively. n.s. denotes not significant.



spite of this, there have been conflicting results regarding the changes in cough reflex sensitivity to capsaicin among human chronic smokers.<sup>3–7,16</sup> It is widely reported that capsaicin- and citric acid-induced cough reflex sensitivities in current smokers were significantly lower than those of never-smokers. In the present study, we demonstrated that capsaicin-induced cough reflexes in current smokers were significantly lower than those of never-smokers. However, the underlying mechanisms for the down-regulation of cough reflex sensitivity to capsaicin in current smokers are not fully understood.

Although cough is usually referred to as a reflex controlled by the brainstem, coughing can also be controlled via the higher cortical center and be related to cortical modulations.<sup>29</sup> Therefore, depression of the cough reflex could be due to both the cortical facilitatory pathway for cough and the medullary reflex pathway. Since the urge-to-cough is a brain component of the cough motivation-to-action system,<sup>30</sup> depressed urge-to-cough in capsaicin-evoked cough suggests impairment of motivation and the reward pathway for cough, which is located in the supra-medulla.

Unpleasant respiratory sensations such as the urge-to-cough can be the result of sensory activation of subcortical and cortical neural pathways. Some of these pathways are shared across respiratory modalities while activation of some neural areas are modality specific.<sup>31</sup> Brain imaging studies have shown that ascending cough sensory information recruits neuronal activity in a variety of higher order brain regions, including primary sensory, insula, cingulate, premotor, motor, and orbitofrontal cortices.<sup>32,33</sup> These regions likely contribute to the perceptual awareness of airway irritation by TRPV1 and TRPA1 stimulations, and the resulting cognitive, emotional, and behavioral consequences that arise (unpleasantness, anxiety, spatial awareness, and the motivational drive to respond). In addition, since it has been reported that nicotine acts on central neural mechanisms as an anxiolytic,<sup>15</sup> primary sensory, insula, cingulate, premotor, motor, and orbitofrontal cortices could be suppressed by nicotine. It must still be clarified whether the central processing of afferent neural input from TRPA1 on airway nerve ending is consistent with that from TRPV1.

In the present study, we demonstrated that cinnamaldehyde-evoked cough motor responses and urge-to-cough were similar in never-smokers and current smokers (Figure 3a–c). These phenomena have not been reported in other studies of induced cough and urge-to-cough in smokers.<sup>16,17</sup> Reflex cough is initiated by sensory stimulation from peripheral afferents.<sup>34–36</sup> These afferents are known to project to the cerebral cortex, affective and cognitive centers.<sup>31,33</sup> Thus, since nicotine can play a role in the central neural mechanism for cough, it seems to facilitate the down-regulation factor of the cough reflex in response to cinnamaldehyde due to the depression of the cerebral cortex, and the affective and cognitive centers.

However, although there are various chemical components among the approximately 5000 constituents of cigarette smoke, recent studies have found that nicotine, reactive oxygen species, and  $\alpha,\beta$ -unsaturated aldehydes included in cigarette smoke directly activate TRPA1 but not TRPV1 channels.<sup>11–13</sup> In addition to these findings, Talavera

et al. have reported that TRPV1 is inhibited by nicotine.<sup>12</sup> Taken together, it is indicated that TRPA1 is more likely to cause up-regulation of peripheral afferent activity for cough by smoking.

Stimulation of these afferents is also the first step in eliciting the cough sensation that precedes the motor action of cough. Thus, since the cough reflex in current smokers can be modified by down-regulation of the central neural facilitatory pathway for cough, the dissociation between the enhanced peripheral afferent activity and the depression of central neural facilitatory pathway is related to be results of the cinnamaldehyde-induced cough responses that are similar in never-smokers and current smokers.

It has not been fully elucidated whether there is cooperation between TRPV1 and TRPA1 channels. The dependence of TRPA1 on  $\text{Ca}^{2+}$  may result in the desensitization of TRPA1 channels by decreased intracellular  $\text{Ca}^{2+}$  in the locale of desensitized TRPV1 channels.<sup>38</sup> However, since the selective TRPV1 antagonist could not inhibit the cough response induced by the TRPA1 selective agonist in animals, whether this sort of cooperation exists in generating a cough reflex has yet to be clarified.<sup>8</sup>

Finally, in the present study, our data show that the log  $\text{C}_5$  value for cinnamaldehyde in all subjects is a relatively high concentration compared with that of capsaicin. Several evidences have been presented indicating that expression of TRPA1 restricts a small sub-population of neurons involved in cough.<sup>39,40</sup> As shown by existing results, including experimental studies (naïve animals),<sup>8,9</sup> TRPA1 activation initiates cough that is relatively modest compared to the cough initiated by the TRPV1 activation (increased concentration TRPA1 agonist vs. TRPV1 agonist). Taken together, this is likely due to the lower efficacy of TRPA1 stimulation to induce sustained activation of the cough-triggering airway afferent nerves.

The present study shows that smoking has differential effects on cough responses to TRPV1 and TRPA1 stimulations. Smoking inhibits TRPV1-induced but not TRPA1-induced cough, suggesting that the effect of smoking status on cough reflex sensitivity is dependent on tussigen. Cigarette smoking is a risk factor for diverse respiratory diseases.<sup>37</sup> Our study suggests that we should pay attention to the smoking status and the receptor responsible for challenged tussigen in order to assess the cough reflex sensitivities in respiratory disease.

## Conflict of interest statement

All the authors declare that they have no competing interests that might be perceived to influence the results and discussion reported in the present manuscript.

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